[ORIGINAL RESEARCH]

A Novel Glycinate-based Body Wash

Clinical Investigation Into Ultra-mildness, Effective Conditioning, and Improved Consumer Benefits

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ABSTRACT

Objective: To assess the properties of a novel body wash containing the mild surfactant glycinate. **Design:** Biochemical and clinical assays. **Setting:** Research laboratories and clinical sites in the United States and Canada. **Participants:** Women 18 to 65 years of age (cleansing efficacy); male and female subjects 26 to 63 years of age with mild or moderate dryness and erythema (leg-controlled application test); subjects 5 to 65 years of age with mild-to-moderate eczema (eczema compatibility); and women 18 to 64 years of age (home use). **Measurements:** Assessments across studies included colorimetric dye exclusion to assess skin damage potential (corneosurfametry), efficacy of cosmetic product removal from skin, change from baseline in visual dryness, change from baseline in Eczema Area and Severity Index, and self-perceived eczema attributes and self-reported product preference. **Results:** The glycinate-based cleanser demonstrated mildness to skin components when evaluated in a corneosurfametry assay. Short-term use under exaggerated wash conditions in subjects with dryness scores <3 and erythema scores <2 (both on a 0–6 scale) indicated an initial reduction in visual dryness. In subjects with eczema, normal use resulted in significant improvements ($P<0.05$) at Week 4 compared with baseline in skin dryness (change from baseline = -0.73), rash (-0.56), itch (-0.927), tightness (-0.585), and all eczema (-0.756). The glycinate-based body wash removed 56 percent of a long-lasting cosmetic foundation from skin compared with less than 30 percent removed by two competitive products tested. The glycinate-based body wash was preferred over a competitive mild cleansing product overall. **Conclusion:** The patented glycinate-containing body wash demonstrated better product mildness and patient-preferred attributes and clinical benefits.
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Harsh cleansing has numerous clinical consequences for skin, including dryness, increased transepidermal water loss (TEWL), fissuring, flaking, and itch. Surfactant molecules cause product lathering; however, they are also the primary cause of cleansing damage to stratum corneum (SC) lipids and proteins. While it may be impossible for cleansers to have no negative impact on the skin, attempts to formulate cleansers to reduce cleanser-induced damage have been successful. Since the introduction of the synthetic detergent (syndet) bar in the 1950s, research has been focused on designing and producing body cleansers that are both efficacious and less harsh to the skin barrier.[1–3] The relatively recent discovery that the SC is not a "dead" layer of cells, but rather a critically important biochemically active component of the epidermis, has led to an increased effort to develop products that are milder to the SC. Greater understanding of the role of SC components (keratin protein filaments, intercorneocyte lipids, SC enzymes) has informed the formulation of skin cleansers with increasingly mild surfactants. Cleanser surfactants can bind to SC proteins, leading to keratin swelling within corneocytes and subsequent structural damage to the SC as well as damage to and denaturation of key SC enzymes.[4] The emergence of milder, less-alkaline surfactants (e.g., amphoterics) in body cleansers has significantly reduced product harshness by reducing surfactant-induced damage to the proteinaceous components of the SC.[4–6] However,

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improvements in surfactant chemistry and the use of ingredients with reduced protein damage potential have in some cases left lipids susceptible to damage and extraction.

Surfactant micelles readily solubilize lipids, particularly those of similar hydrophobic chain length. Even brief (<1 min) direct exposure of skin to surfactants can result in the removal of SC lipids.[7] Thus, both the protein and lipid components of the SC need to be protected for optimal skin barrier function, and cleansers that are mild to skin should employ a combination of ingredients to minimize damage to the SC.[1,2] Current innovations in skin cleansing have achieved substantial improvements in mildness using this formulation approach.

A COMPREHENSIVE APPROACH TO CLEANSER FORMULATION

In recent years, mild and moisturizing body washes have been developed to include a combination of anionic and amphoteric surfactants (to reduce protein denaturation and skin irritation potential), moisturizing oils, and humectants. There has been a trend toward increasing the concentration of occlusive moisturizers (e.g., petrolatum) to greater than 50 percent in body washes to provide skin occlusion post-wash. However, this strategy does not prevent SC damage during cleansing; it serves primarily to mask damage already done by cleansing. Further increasing petrolatum concentration is associated with decreased cleanser efficacy and formulation practicality; the maximal benefits possible with a high-occlusive strategy may have already been achieved. Very mild skin cleansers have also been developed and typically contain very low surfactant concentrations. These products effectively limit the potential for surfactant-induced SC damage, but are low or poorly foaming, which may limit consumer acceptance or compliance with regular use. Other products deliver consumer benefits, but do not perform optimally to protect the SC during cleansing.

The key insight from research is that protection of both lipids and proteins is required to effectively minimize cleanser-induced damage to the structure and function of the SC. This insight has led to an understanding of the interaction between cleansing surfactants and the skin, leading to the development of a cleanser that prevents the damage inherent in surfactant use, includes ingredients that proactively promote a healthy skin barrier, and has in-wash properties, such as lathering, consistent with consumer preferences.

Dove® Body Wash with Nutrium Moisture™, first introduced in 2009, was formulated with several ingredients that, in combination, reduce cleansing-induced damage to the SC, aid in moisturization, and replenish the SC with skin-natural lipids.[2,8] The surfactant system in the proprietary cleanser, directly esterified fatty isethionate (DEFI), includes the mild surfactant sodium cocoyl isethionate presaturated with stearic acid, a fatty acid naturally found in the SC. The stearic acid in the DEFI has two significant functions—to act as a buffer against solubilization of the SC lipids into surfactant micelles and to deposit stearic acid into the SC to replace the fatty acids lost during normal cleansing. The amount of deposited stearic acid is equivalent to that extracted during washing and is incorporated into the SC 10 layers deep.[8] Because fatty acids are readily extracted during surfactant use, their replenishment during cleansing is important for barrier protection.[9,10] This approach of mild surfactant combined with skin-identical lipids is enhanced by the inclusion of triglyceride oils (e.g., sunflower, soybean), which can bind to SC proteins, impeding surfactant access and providing protection from cleanser-induced damage. The visual and clinical benefits of this DEFI-based mild and moisturizing cleansing technology have been seen in patients with eczema, winter xerosis, and patients with skin of color.[2,11,12]

As the knowledge of skin biology, the interaction of cleansing ingredients with SC components, and the clinical consequences of cleansing continues to expand, it has become more feasible to design products that effectively cleanse skin while minimally disrupting key biological processes. The sequential use of *in vitro*, *ex vivo*, and *in vivo* testing has facilitated a more detailed analysis of novel cleansing products, allowing for greater insight into the impact of product use on key SC parameters (e.g., protein and lipid damage potential, permeability, barrier integrity, clinical dryness). The authors have developed a comprehensive set of parameters based on this knowledge, prioritized below:

- Ultra-mild surfactant with low potential for damage to SC proteins and lipids;
- Inclusion of ingredients that enhance surfactant mildness and replenish the SC;
- Formulation with appropriate lathering and rinsing properties to meet consumers' needs;
- Comprehensive analytical methods (*in vitro*, *ex vivo*, and *in vivo*) to analyze product efficacy and comparative benefits.

This strategic approach has led to identification of a DEFI-based body wash that demonstrates improved, further-enhanced mildness and overcomes a significant challenge by meeting both clinical and consumer needs.

GLYCINATE—A NEW DEVELOPMENT IN BODY WASH SURFACTANTS

The amount of mild surfactant used in a cleanser is limited by the decline of in-use properties that occurs above a certain concentration; mildness continues to increase, but at the expense of consumer-desired lathering ability. It has been demonstrated that an effective cleanser need not foam extensively. Cleansing with water alone has been shown to remove approximately 65 percent of dirt and oils from the skin, suggesting that only a small amount of surfactant active is required to cleanse the skin.[13] Despite this, users of cleansing products nearly universally require the presence of lather to confirm cleansing efficacy.[14,15] Body cleansers with exceptionally mild properties (mild surfactants and/or very low surfactant concentrations) can be a challenge in

real-world use because they are low lathering. A more viable strategy may be to include the use of ultra-mild surfactants that cause minimal damage to the SC that also have the high-lathering characteristics users desire.

The authors sought to identify a surfactant with a low potential for damage to SC components to combine with the mild DEFI-based surfactant, replenishing lipids, and moisturizing ingredients for inclusion into a new body wash. This product was also designed to meet consumers' need for a high-lathering product while maintaining significant mildness to skin. This approach is suited to meet the evolving needs of basic skin care consumers while formulating products that do less harm to the skin by using mild surfactants, including moisturizing ingredients (e.g., soybean oil) that are readily absorbed by the skin and delivering stearic acid directly to the SC to replenish fatty acids lost during normal cleansing.

Sodium N-cocoyl glycinate (subsequently referred to as glycinate; Figure 1) is an amino acid–based surfactant derived from natural coco fatty acid and the amino acid glycine. Glycine is the smallest of the naturally occurring amino acids; accordingly, the charged head group on glycinate is significantly smaller than on many other surfactants, including sodium lauryl ether sulfate (SLES). This small size facilitates production of smaller surfactant micelles and the generation of a creamy lather during use. Although glycinate possesses the intrinsic lathering ability common to most anionic surfactants, it is unique in that it demonstrates a low potential for SC damage. *In vitro* studies indicate that cleansers formulated with glycinate are significantly less damaging to SC proteins than those including other anionics (data not shown). Because of the properties associated with glycinate surfactant, it has to-date been used primarily in facial cleansers. The mildness and in-use attributes of glycinate favor its expansion to use in body washes as an improvement in mild and moisturizing body cleansing.

However, the use of glycinate alone as a cleanser surfactant in body washes is impractical, in part due to cost implications. Furthermore, a body cleanser that minimizes damage to both lipids and proteins provides further benefits to skin than simply mild surfactants alone. Thus, the authors developed a novel body wash formulation that includes glycinate in combination with the clinically proven mild DEFI surfactant system. The combination is further strengthened by the ability of glycinate to function synergistically with more traditional surfactants, specifically by increasing lathering properties to a degree that exceeds a simple additive effect (data not shown). In addition, this new formulation allows for a reduction in SLES, resulting in a reduction in the total concentration of surfactants present, further enhancing product mildness.

In this article, the authors report on the clinical and cleansing efficacy of a novel mild and moisturizing body wash for sensitive skin that includes glycinate in combination with the previously described lipid-saturated surfactant system, DEFI, formulated within a new body cleansing product.[2]

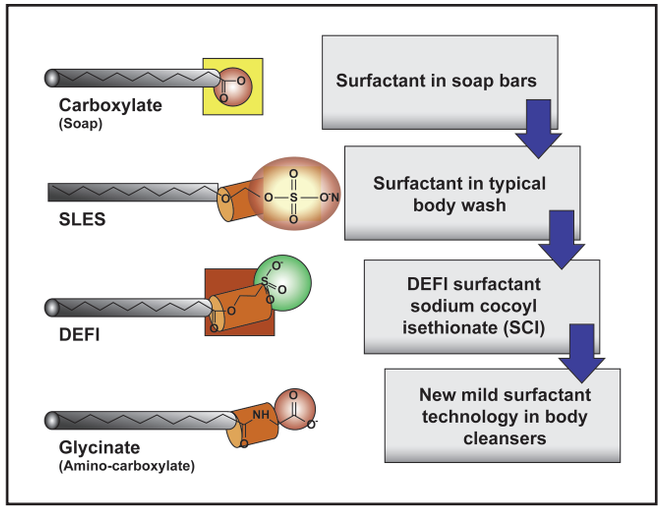

Figure 1. Surfactant molecules used in skin cleansers

METHODS

Because cleanser ingredients can directly interact with SC components (e.g., lipids, enzymes, keratins) and affect basic skin mechanisms, the novel glycinate-containing body wash was investigated using a comprehensive set of techniques. A series of experimental designs was created to validate its performance on key elements of body wash design. These key elements included mildness, cleansing, moisturization, skin sensitivity, and consumer attributes. *In vitro* zein solubility assays and *ex vivo* corneosurfametry were utilized to assess the mildness of the glycinate-containing body wash.

Zein solubility assay. Zein solubilization by surfactants is a commonly used method to predict the protein damage (denaturation) potential of a surfactant. Corn-derived zein protein is a model protein that is highly insoluble in water. The surfactant-induced denaturation and solubilization of zein are associated with the surfactant's skin irritation potential. In this study, zein dissolution by surfactant solutions was used as an indicator of protein denaturation potential of the surfactant. Zein powder was mixed with 5wt% surfactant solution for 24 hours. Solutions were then filtered using a nylon membrane. Dissolved zein was separated from the undissolved materials and the in-solution zein concentration was determined using UV absorbance.

Corneosurfametry. Corneosurfametry is a rapid and noninvasive *ex vivo* assay that generates quantifiable visualization of cleanser impact on actual human SC. Although not commonly reported in the literature as a screening assay for body cleansers but for general surfactants and shampoos, it has proven to be an important tool for obtaining a comprehensive view of a cleanser's mildness profile, including relative SC damage potential compared with other products. This test is also used as a predictive model for *in vivo* testing selection.

D-Squame® (CuDerm Corporation, Dallas, Texas) tape strips were applied to subjects' forearm skin, removed,

and then placed into a 10% (w/w) solution of sample body wash product. Following a10-minute incubation period at room temperature, the strips were rinsed with water and allowed to dry at room temperature for 30 minutes. Damage to the SC was determined by immersing the dried tape-stripped skin into a basic fuchsin toluidine blue dye solution (PMS Fungal/Tzanck Stain, Delasco Dermatologic Lab & Supply, Inc., Council Bluffs, Iowa) for three minutes at room temperature, rinsing with water, then air drying for approximately 30 minutes. An irritant material will induce a strong staining of the skin sample, while a mild solution will induce minimal staining. Stain binding was measured by colorimeter (Konica Minolta CR-400 Chroma Meter, Sensing Singapore Pte, Ltd) performed at three distinct sites per D-Squame tape. The colorimetric index of mildness (CIM) was calculated as follows:

$CIM = L^* - C^*$, where $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$

The higher the CIM, the milder the product. A large set of products was tested by corneosurfametry, and based on test outcome, the best performers were included in the *in vivo* forearm-controlled application test (FCAT) to show mildness.

Once product attributes were validated by *in vitro* and *ex vivo* methods, the authors conducted a series of clinical assays to determine cleansing efficacy, assess moisturization benefits, and confirm suitability for sensitive skin. Because a primary goal of this new technology is to improve clinical mildness by minimizing damage to proteins and lipids and to deliver moisturization, the glycinate-based body wash was evaluated in a leg-controlled application test (LCAT) to assess moisturization efficacy as determined by skin barrier function and clinical dryness. Additionally, atopic dermatitis (AD)/eczema is a highly prevalent condition frequently associated with skin dryness and irritation. For this reason, the authors investigated the compatibility of the glycinate-based body wash in adults with mild/moderate eczema.

Cleansing efficacy (cosmetic product removal) test. A standard cosmetic removal assay was employed to evaluate the efficacy of select cleansers in cleansing the skin of a common and difficult-to-remove soil. This was assessed in a randomized, double-blind study in 20 Caucasian women aged 18 to 65 years. Topical moisturizers, sunscreens, and washing devices were not permitted within three days of starting the study or during the study. Prior to cosmetic application, the subjects prepared their forearm skin by creating a lather with a sensitive skin syndet bar, washing for 30 seconds, rinsing with water for 15 seconds, then patting the area dry. Liquid foundation makeup was applied to a 3.5x2.5cm area, allowed to dry for 10 minutes, then washed off with test body wash products. Chroma Meter CR-10 measurements were taken at baseline (following pre-wash) after applied makeup was dried and 10 minutes post-makeup removal. Percent of makeup removal was calculated using the following:

$[(B\text{-}C) / (B\text{-}A)]^* 100$, where:

$B - C = [(L^*_{makeup} - L^*_{washed})^2 + (a^*_{makeup} - a^*_{washed})^2 + (b^*_{makeup} - b^*_{washed})^2]^{1/2}$

$B - A = [(L^*_{makeup} - L^*_{baseline})^2 + (a^*_{makeup} - a^*_{baseline})^2 + (b^*_{makeup} - b^*_{baseline})^2]^{1/2}$

Statistical analysis was conducted using a 2-way analysis of variance and Tukey multiple comparison adjustment. Significance was defined as P=0.05 using a 2-tailed hypothesis.

Moisturization efficacy—LCAT. A moisturizing body wash should provide effective skin conditioning as well as ultra-mild surfactants to the skin. The DEFI-glycinate formulation includes a combination of ingredients known to be effective skin conditioners. A standard LCAT was conducted in 37 women and 13 men (aged 26 to 63 years). The study included a five-day conditioning phase followed by a five-day product (glycinate-containing body wash) application phase. At baseline, subjects had dryness scores <3.0 (on a 0–6 scale where 0=no dryness and 6=severe cracking, large scales) and erythema scores <2.0 (on a 0–6 scale where 0=no erythema and 6=severe redness). Subjects used unscented sensitive skin syndet bars for all cleansing during the conditioning phase and were restricted from using any moisturizers, bath oils, or other skin products on their lower legs. During the product application Days 1 to 4, subjects completed two daily wash sessions approximately 3 to 4 hours apart; a single wash was performed on Day 5. Instrumental (Skicon and Corneometer) and expert-assessed evaluations were performed daily during the product application phase. This study was conducted in January 2012 in Winnipeg, Canada. Data were reported as change from baseline (CFB).

Eczema compatibility study. Atopic dermatitis, or eczema, is a relatively common chronic inflammatory skin disease characterized by dry, pruritic skin lesions frequently accompanied by xerosis. Traditional management strategies for eczema include use of emollients/moisturizers, anti-inflammatory agents, antibiotics, and immunosuppressive agents (e.g., corticosteroids). Because minimization of triggers and prevention of skin irritation/inflammation are keys to controlling the disease, mild and moisturizing cleansers are an important component of the management of eczema.

A double-blind monadic study was conducted to assess the compatibility of the glycinate-containing body wash with mild-to-moderate eczema in adult (18–65 years old; n=23) and pediatric (5–14 years old; n=18) subjects. Subjects with mild-to-moderate atopic eczema with at least two active lesions (accessible for clinical and instrumental evaluation) were enrolled in the study. All subjects refrained from using moisturizers or other skin care products. Exceptions included prescribed eczema treatments, provided they had been used for a minimum of three months prior to study initiation. Prescribed moisturizers were not permitted on the days of study visits until after evaluations were completed. At Week -2 (subject screening), Week 0 (baseline), and Weeks 2 and 4, subject questionnaires were administered and Eczema Area and Severity Index (EASI) scores were calculated by a dermatologist. Analyses for all assessments were reported as average and CFB; determination of significance was

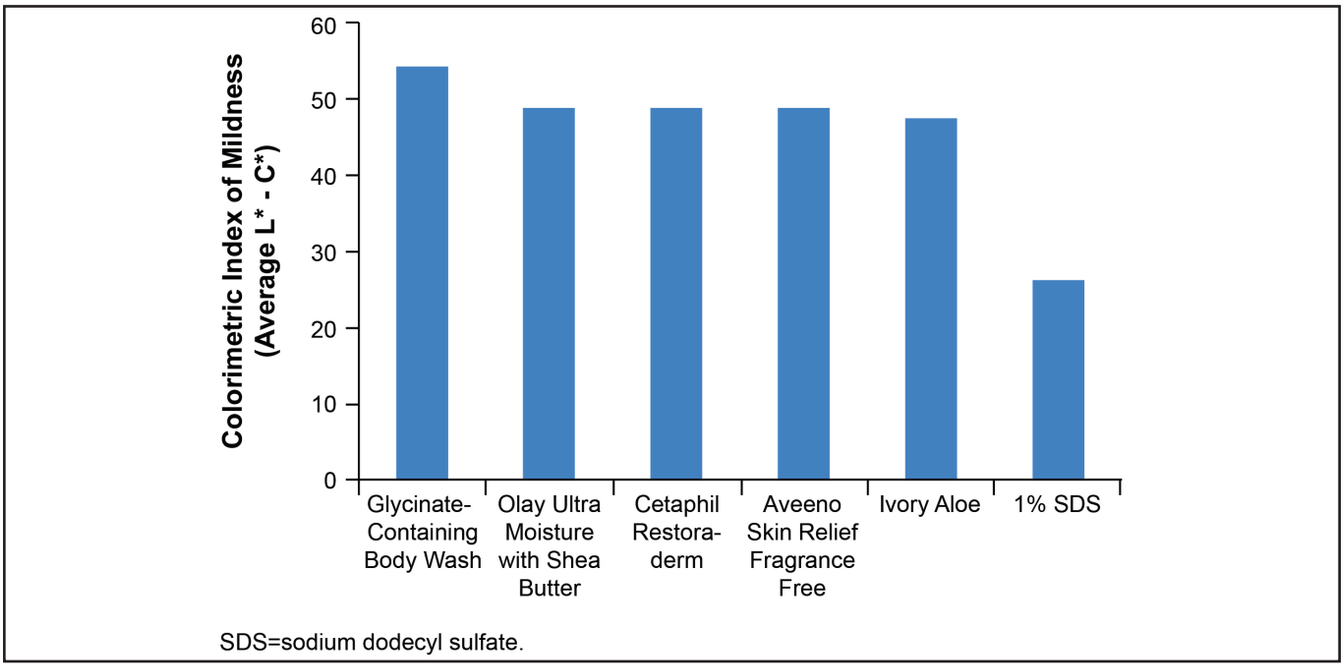

Figure 2. *Ex vivo* corneosurfametry analysis of body wash–induced damage to stratum corneum tissue

measured at the 0.05 *P*-value using parametric statistics.

Home-use study. The authors evaluated the glycinate-containing body wash across a variety of consumer attributes and compared its performance to an existing leading mild body cleansing product. This approach to product testing (confirming the science via progressive testing and assessing consumer perspectives on product attributes) provides a comprehensive view of how a new approach to cleanser design can translate into a clinically beneficial and consumer-relevant product. Women aged 18 to 64 years (N=307), most with self-described sensitive skin, were enrolled in a two-week, normal-home-use study. Each participant used one body wash product for seven consecutive days, then used a second product for another seven days. All participants received the glycinate-containing body wash; each group also received 1 of 2 currently marketed moisturizing body washes. Product assessments were made after each seven-week cleansing period.

RESULTS AND DISCUSSION

***In vitro* and *ex vivo* assays of skin damage potential.** To determine the degree of mildness of the DEFI-glycinate formulation, *in vitro* and *ex vivo* experiments were conducted with fully formulated body washes. In the zein solubility assay, the glycinate-containing body wash caused significantly less protein damage than did several leading moisturizing body washes (data not shown).

Corneosurfametry results show that the glycinate-containing body wash excluded dye to a similar extent as water alone and to a greater degree than several other tested body washes, including a previously available proprietary body wash (Figure 2). The CIM for the glycinate-containing product was approximately 56 compared with the harsh surfactant 1% sodium dodecyl sulfate (SDS), which had a CIM of 26 and significant dye incorporation. The glycinate-containing body wash resulted in a higher CIM than most products tested, thus demonstrating greater mildness to SC components.

Cleansing efficacy. One potential consequence of achieving ultra-mildness in a cleanser is loss of cleansing efficacy. To assess this, the authors investigated the DEFI-glycinate formulation in a makeup removal study. The glycinate-containing body wash removed approximately 56 percent of a long-lasting foundation product; this was significantly greater than the removal observed with two other gentle liquid cleansers (28 and 18%, respectively) (Figure 3).

Moisturization efficacy. Dryness is a common condition frequently managed by the use of topical moisturizers, creams, and oils. We have previously demonstrated improvements in clinical dryness achieved with regular use of the mild and moisturizing DEFI-based cleanser; we hypothesized that the DEFI-glycinate formulation would also improve skin dryness.[2,12] Changes in skin dryness with body wash use were assessed using controlled application tests on arms and legs. After five days of use, the DEFI-glycinate body wash resulted in improvement in visual dryness on leg skin compared with baseline (Figure 4). Clinical dryness is closely associated with the integrity of the lipid bilayer, thus the observed improvements were likely due to a combination of lipid and protein protection during cleansing. In addition, the stearic acid from the new DEFI-glycinate formulation was

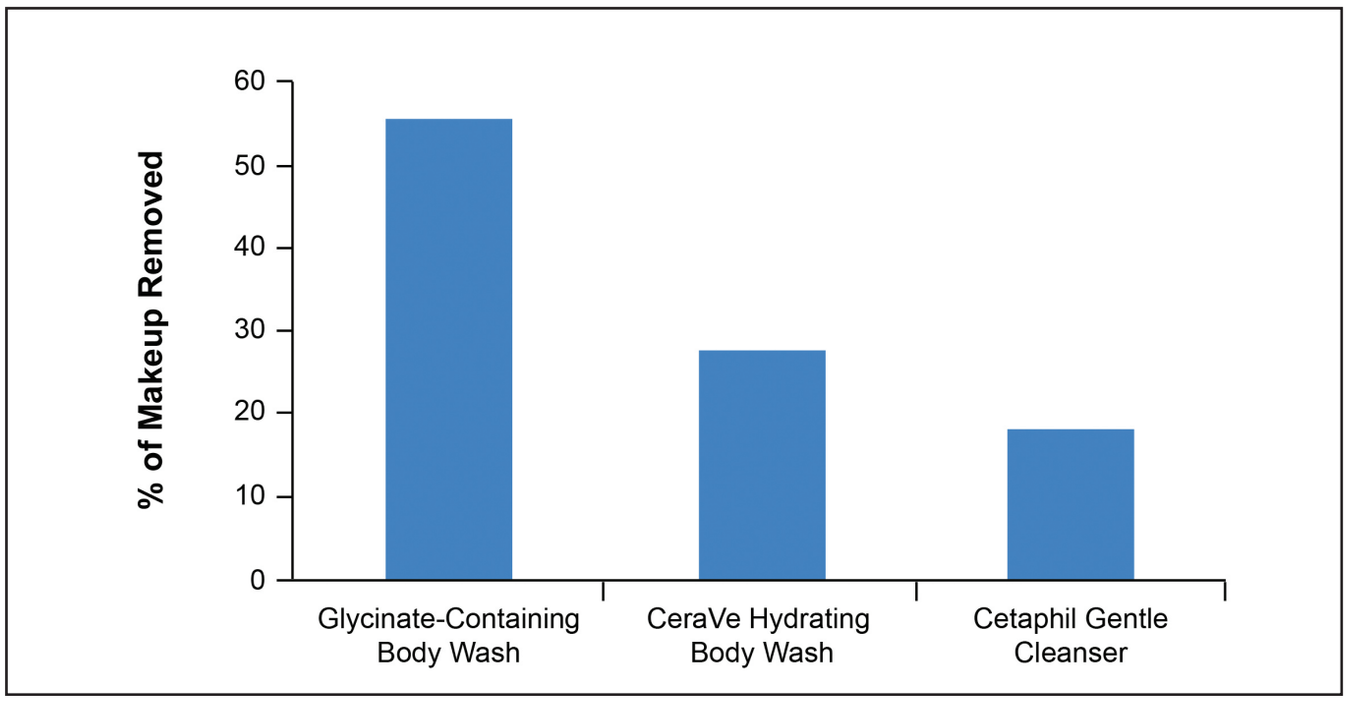

Figure 3. Cleansing efficacy of glycinate-containing body wash in removal of foundation makeup

effectively deposited during wash and subsequently incorporated into the SC (data not shown). Stearic acid incorporation provides near-immediate compensation for any surfactant-mediated extraction from the SC by replenishing a fatty acid species critical to barrier maintenance. This may also play a role in SC barrier homeostasis by adding to the pool of precursor molecules required for endogenous lipid synthesis (e.g., ceramides).

Compatibility with atopic dermatitis. Both patient groups (adults and children) experienced a significant reduction in EASI scores compared with baseline (Figure 5). This improvement was maintained over the four-week study period. Multiple symptoms are frequently experienced by patients with eczema, so a self-perception questionnaire was included to assess tactile aspects of the condition. Patients using the glycinate-containing body wash reported significant improvements ($P<0.05$) at Week 4 compared with baseline in skin dryness (CFB = -0.73), rash (-0.56), itch (-0.927), tightness (-0.585), and all eczema (-0.756).

Consumer preference study. A persistent challenge in mild body cleanser development is ensuring that the final body wash formulation has in-wash properties that are satisfactory to the product user. For most cleansing products (including body washes), foaming and rinse-off properties dictate much of the perception of efficacy. A two-week home-use study was conducted to determine in-use properties of the glycinate-containing body wash. Participants used the novel body wash and a competing brand of sensitive skin body wash, each for seven days. Product attributes were assessed after a week of regular use to determine if the ultra-mild glycinate-containing product met the in-use needs of individuals with sensitive skin.

The glycinate-containing body wash was preferred to the other mild cleanser in multiple categories pertaining to product lathering, scent, and overall preference (Table 1). Additional attributes, including moisturization, nourishment, and skin feel, were numerically higher with the glycinate-containing body wash than with the comparator product, although these differences did not reach statistical significance.

CONCLUSION

These findings indicate that the glycinate-containing body wash does not compromise the in-use properties (e.g., lathering) as is often the case with body washes designed to be mild to sensitive and/or diseased skin. For individuals with relatively normal skin, use of mild and moisturizing body washes can preserve barrier function and promote overall SC health. In individuals with disorders or diseases in which the SC barrier is chronically impaired, the need for mild cleansers that deliver moisturization is even greater. The glycinate-containing body wash demonstrated superior mildness and in-use properties associated with user compliance.

Additional physiological skin benefits of a surfactant based on glycine, a significant (~30%) component of human collagen, remain to be elucidated, but are a potential avenue of future study. Regular use of a mild and moisturizing cleanser formulated with a mild surfactant system (mild surfactant character in combination with lipid pre-saturation), effective moisturizing ingredients, and replenishing fatty acids, has demonstrated benefits in reducing cleanser-

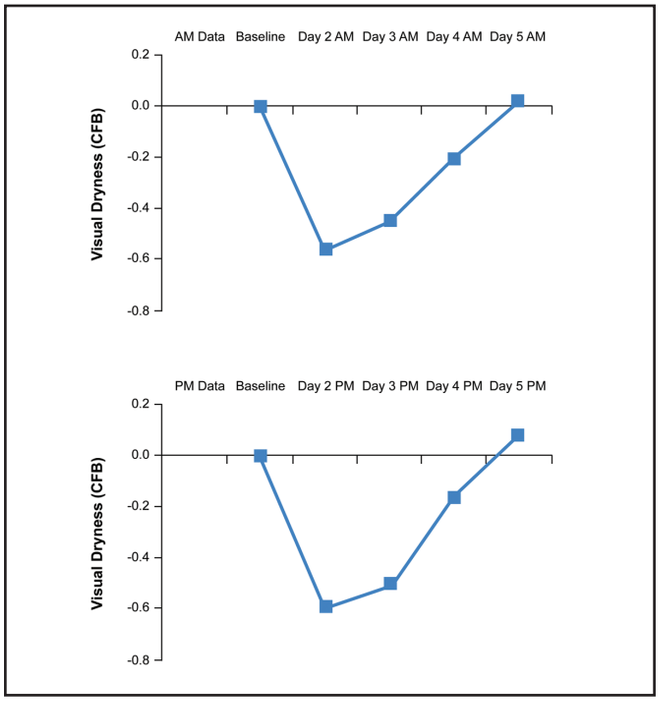

Figure 4. Short-term changes in visual dryness with glycinate-based cleanser use. Data represent change from baseline. CFB=change from baseline.

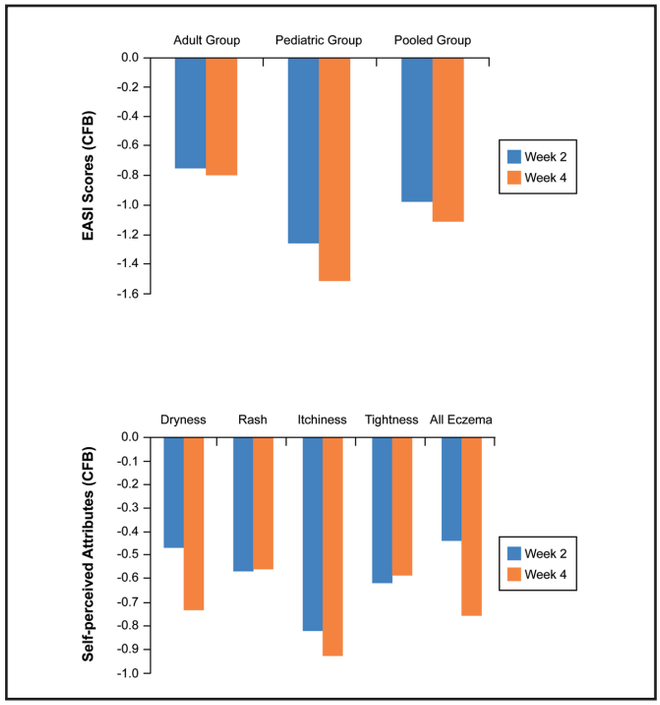

Figure 5. Changes in EASI and self-perception with glycinate-based cleanser use. Data represent change from baseline. CFB=change from baseline.

TABLE 1. Consumer home-use study of the glycinate-containing body wash and a Cetaphil cleanser. Data represent the percentage of subjects who preferred either test product

ATTRIBUTE	PREFERS DOVE GLYCINATE-CONTAINING BW (%)	PREFERS CETAPHIL CLEANSER (%)	NO PREFERENCE (%)
Overall preference	53	35	12
Speed of lather	38	25	37
Richness/creaminess of lather	50	20	30
Product consistency	50	18	31
Cleansing efficacy	25	21	54
Moisturizing effect	36	27	37
Nourishment	30	23	46
Skin feel during use	28	21	51
Daily use	38	23	39

induced damage to the SC.[1,2] A comprehensive biochemical and clinical analysis of cleanser ingredients and formulated products has led to the successful incorporation of the ultra-mild glycinate surfactant into a novel body cleanser. This approach improves upon existing technology by addressing both the clinical need for skin barrier protection during cleansing and the consumer need for products with desired aesthetic properties. With continued innovation in both the biochemistry of cleanser ingredients and the understanding of fundamental skin biology, cleanser technologies will continue

to advance, leading to products with minimal impact on skin barrier integrity.

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